

**APPLICATION**  
**FOR**  
**UNITED STATES LETTERS PATENT**

**TITLE:           APORPHINE AND OXOAPORPHINE COMPOUNDS**  
**AND PHARMACEUTICAL USE THEREOF**

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# **APORPHINE AND OXOAPORPHINE COMPOUNDS AND PHARMACEUTICAL USE THEREOF**

## **Cross-reference to related applications**

- [0001]** This application claims priority from Taiwanese Application No. TW92107780 filed on April 4, 2003, Chinese Application No. 03137381.X filed on June 19, 2003, and PCT Application No. PCT/CN03/00477 filed on June 19, 2003.

## **Background of Invention**

### **Field of the Invention**

- [0002]** The present invention relates to compounds for treating ischemic diseases, more particularly to aporphine and oxoaporphine compounds that can be used to prevent or treat ischemic diseases based on a mechanism of maintaining or increasing the endothelial nitric oxide synthase (eNOS).

### **Background of the Invention**

- [0003]** With the progress of society and the advance in sciences and technology, life expectancy gradually increases. Many people now suffer from various diseases due to old age, diet, obesity, lack of exercise or living under stress. Among these diseases, ischemic diseases are among the main causes of death and physical impairment. Ischemic diseases have a major impact and cause substantial loss to people, family, society and the state. Therefore, it is important to find agents to prevent the ischemic diseases.
- [0004]** Among various ischemic diseases, ischemic stroke has a high rate of morbidity, mortality, handicap and recurrence, and it is a common disease among middle-aged and old people. Occlusion of the extracranial or intracranial arteries, which supply blood to the brain, may cause brain ischemia and hypoxemia, resulting in a series of acute clinical symptoms (i.e. ischemic stroke). If blood supply cannot be restored in time, nerve cells, glial cells, vascular endothelial cell and smooth muscle cells will die, leading to cerebral infarction and cerebral thrombosis.

**[0005]** For the ischemic stroke, the thrombolytic agent, tissue plasminogen activator, is the only medicament that can re-open occluded blood vessels. Tissue plasminogen activator is also the only medicament that has been approved for treating thromboembolic stroke by the Food and Drug Administration in the U.S., and it was also approved by the Health Administration in Taiwan in 2001. However, because patients may suffer cerebral hemorrhagic complication after using a thrombolytic agent, this agent is strictly restricted in its use in a therapeutic window, i.e. "critical period." This drug should be injected intravenously within 3 hours after stroke, or injected intra-arterially with the aid of cerebral angiography to directly lyse the thrombus within 6 hours after stroke.

**[0006]** Other conventional blood vessel unblocking agents, such as anticoagulants and platelet aggregation inhibitors, can only prevent continued thrombus formation, but cannot lyse the thrombus to re-open occluded blood vessels. In addition, brain tissue protecting agents (such as Piracetam) also offer a promising therapy for thrombosis. However, these agents also need to be administrated within 6 hours after stroke to achieve a significant effect.

**[0007]** Recent studies show that blood clot dissolving agents can trigger the production and release of oxygen free radicals, platelet activating factor (PAF), and excitotoxic neurotransmitter, such as glutamate, which may act as N-methyl-D-aspartic acid (NMDA) to stimulate the NMDA receptor and consequently lead to neuronal cell death. Though MK801, an NMDA receptor antagonist, can reduce the injury (or infarction) in ischemia or ischemia-reperfusion brain tissue, the hypothermic effect, suppression of memory and other side effects of this agent prevent it from being used as a therapeutic agent.

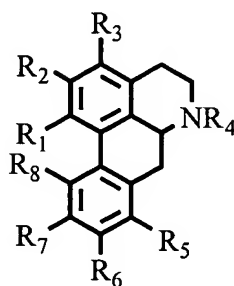
### **Summary of the Invention**

**[0008]** One object of the invention is to provide aporphine and oxoaporphine compounds that can be used in the prevention and treatment of local ischemia. These compounds prevent and treat local ischemic injury by maintaining or increasing endothelial nitric oxide synthase (eNOS) activity.

**[0009]** Another finding of the present study is that aporphine and oxoaporphine compounds can dilate blood vessels and are more effective in opening up blocked vessels

than conventional clot dissolving agents. As compared with MK801, these aporphine and oxoaporphine compounds will not cause memory loss or hypothermic side effects when they are used to treat ischemic diseases. Therefore, these compounds can provide better therapeutic index.

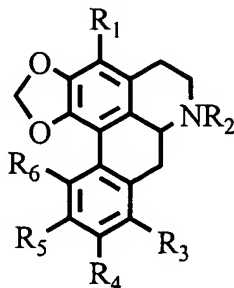
**[0010]** To achieve the above-described objectives, some embodiments of the present invention provide aporphine and oxoaporphine compounds for the prevention and treatment of local ischemic diseases, by maintaining or increasing endothelial nitric oxide synthetase (eNOS) activity. A composition of the invention includes an effective amount of a compound having the following structure I:



I

where  $R_1$ ,  $R_2$ ,  $R_6$  and  $R_7$  are each selected from H, OH, O-acyl, OMe, OEt,  $O^iPr$  and  $O^tPr$ ,  $R_3$  and  $R_5$  are each selected from H, OH, O-acyl, OMe, F, Cl, Br,  $NH_2$ ,  $NO_2$  and CN;  $R_4$  is selected from allyl and  $C_nH_{2n+1}$ ,  $n \geq 0$ ; and  $R_8$  is selected from H, OH, and OMe.

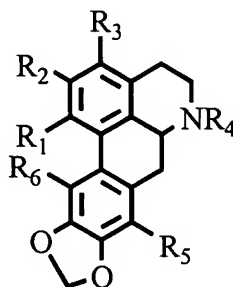
**[0011]** Some embodiments of the present invention provide aporphine and oxoaporphine compounds for the prevention and treatment of local ischemic diseases. These compounds function by maintaining or increasing endothelial nitric oxide synthetase (eNOS) activity. A composition of the invention includes an effective amount of a compound having the following structure II:



## II

where  $R_1$  and  $R_3$  are each selected from H, OH, O-acyl, OMe, F, Cl, Br,  $NH_2$ ,  $NO_2$  and CN;  $R_2$  is selected from allyl and  $C_nH_{2n+1}$ ,  $n \geq 0$ ;  $R_4$  and  $R_5$  are each selected from H, OH, O-acyl, OMe, OEt,  $O^nPr$ , and  $O^iPr$ ; and  $R_6$  is selected from H, OH, O-acyl, and OMe.

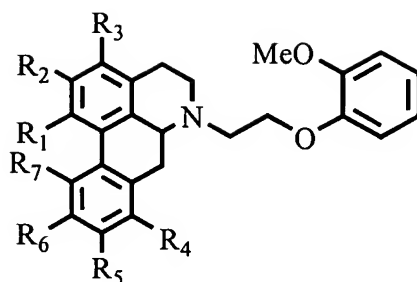
**[0012]** Some embodiments of the present invention provide aporphine and oxoaporphine compounds for the prevention and treatment of local ischemic diseases. These compounds function by maintaining or increasing endothelial nitric oxide synthetase (eNOS) activity. A composition of the invention includes an effective amount of a compound having the following structure III :



## III

where  $R_1$  and  $R_2$  are each selected from H, OH, O-acyl, OMe, OEt,  $O^nPr$ , and  $O^iPr$ ;  $R_3$  and  $R_5$  are each selected from H, OH, O-acyl, OMe, F, Cl, Br,  $NH_2$ ,  $NO_2$  and CN;  $R_4$  is selected from allyl and  $C_nH_{2n+1}$ ,  $n \geq 0$ ; and  $R_6$  is selected from H, OH, O-acyl, and OMe.

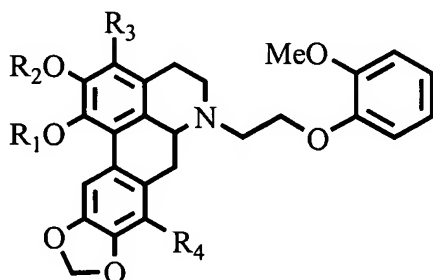
**[0013]** Some embodiments of the present invention provide aporphine and oxoaporphine compounds for the prevention and treatment of local ischemic diseases. These compounds function by maintaining or increasing endothelial nitric oxide synthetase (eNOS) activity. A composition of the invention includes an effective amount of a compound having the following structure IV :



IV

where  $R_1$ ,  $R_2$ ,  $R_5$  and  $R_6$  are each selected from H, OH, O-acyl, OMe, OEt,  $O^nPr$ , and  $O^iPr$ ;  $R_3$  and  $R_4$  are each selected from H, OH, O-acyl, OMe, F, Cl, Br,  $NH_2$ ,  $NO_2$  and CN; and  $R_7$  is selected from H, OH, O-acyl, and OMe.

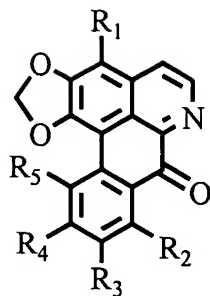
**[0014]** Some embodiments of the present invention provide aporphine and oxoaporphine compounds for the prevention and treatment of local ischemic diseases. These compounds function by maintaining or increasing endothelial nitric oxide synthetase (eNOS) activity. A composition of the invention includes an effective amount of a compound having the following structure V :



V

where  $R_1$  and  $R_2$  are each selected from H, acyl, Me, Et,  $^nPr$ , and  $^iPr$ ;  $R_3$  and  $R_4$  are each selected from H, OH, O-acyl, OMe, F, Cl, Br,  $NH_2$ ,  $NO_2$  and CN.

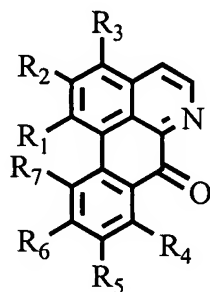
**[0015]** Some embodiments of the present invention provide an aporphine and oxoaporphine compounds for the prevention and treatment of local ischemic diseases. These compounds function by maintaining or increasing endothelial nitric oxide synthetase (eNOS) activity. A composition of the invention includes an effective amount of a compound having the following structure VI :



VI

where  $R_1$  and  $R_2$  are each selected from H, OH, O-acyl, OMe, F, Cl, Br,  $\text{NO}_2$  and CN;  $R_3$  and  $R_4$  are each selected from H, OH, O-acyl, OMe, OEt,  $\text{O}^n\text{Pr}$ , and  $\text{O}^i\text{Pr}$ ; and  $R_5$  is selected from H, OH, O-acyl, and OMe.

**[0016]** Some embodiments of the present invention provide aporphine and oxoaporphine compounds for the prevention and treatment of local ischemic diseases. These compounds function by maintaining or increasing endothelial nitric oxide synthetase (eNOS) activity. A composition of the invention includes an effective amount of a compound having the following structure VII :

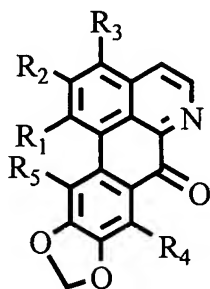


VII

where  $R_1$ ,  $R_2$ ,  $R_5$  and  $R_6$  are each selected from H, OH, O-acyl, OMe, OEt,  $\text{O}^n\text{Pr}$  and  $\text{O}^i\text{Pr}$ ;  $R_3$  and  $R_4$  are each selected from H, OH, O-acyl, OMe, F, Cl, Br,  $\text{NO}_2$  and CN; and  $R_7$  is selected from H, OH, O-acyl, and OMe.

**[0017]** Some embodiments of the present invention provide aporphine and oxoaporphine compounds for the prevention and treatment of local ischemic diseases. These compounds function by maintaining or increasing endothelial nitric oxide synthetase

(eNOS) activity. A composition of the invention includes an effective amount of a compound having the following structure VIII :



VIII

where R<sub>1</sub> and R<sub>2</sub> are each selected from H, OH, O-acyl, OMe, OEt, O<sup>n</sup>Pr and O<sup>i</sup>Pr; R<sub>3</sub>, and R<sub>4</sub> are each selected from H, OH, O-acyl, OMe, F, Cl, Br, NO<sub>2</sub> and CN; and R<sub>5</sub> is selected from H, OH, O-acyl, and OMe.

**[0018]** These compounds can be used in the manufacture of medicaments for treating ischemic diseases in mammal or human beings. The ischemic diseases include ischemic cerebral apoplexy, ischemic cerebral thrombosis, ischemic cerebral embolism, hypoxia and ischemic encephlopathy, ischemic cardiac disease, ischemic enteropathy, and the like.

**[0019]** Compared with the current methods for lysing the infarcted thrombus to open up blood vessels, the compounds of the invention have better therapeutic efficacies by loosening and dilating the blood vessels. There are little side effects in treating the ischemic diseases with the aporphine and oxoaporphine compounds of the invention. These side effects may include, for example, s memory loss, body temperature decreasing.

**[0020]** The present invention also discloses the use of aporphine or oxoaporphine compounds in the prophylaxis or treatment of ischemic diseases, and further discloses the use of aporphine and oxoaporphine compounds in the prophylaxis or treatment of ischemic diseases in mammal and human beings.

**[0021]** The invention also provides pharmaceutical compositions for the prophylaxis or treatment of ischemic diseases. A pharmaceutical composition in accordance with



embodiments of the invention comprises a therapeutically effective amount of aporphine or oxoaporphine compounds and a pharmaceutically acceptable carrier or excipient. One of ordinary skill in the art would appreciate that “an effective amount” refers to an amount sufficient to achieve the prevention or treatment of an ischemic disease. The specific amount will depend on the age, body weight of the patient and the specific ischemia.

[0022] The following examples and the associated figures further describe and demonstrate embodiments of the present invention. These examples are given solely for illustration and are not intended to limit the scope of the invention to these illustrated examples.

#### **Brief Description of the Drawings**

[0023] Fig.1 shows the results of groups I and II in the pharmacodynamic study using a compound in accordance with one embodiment of the invention.

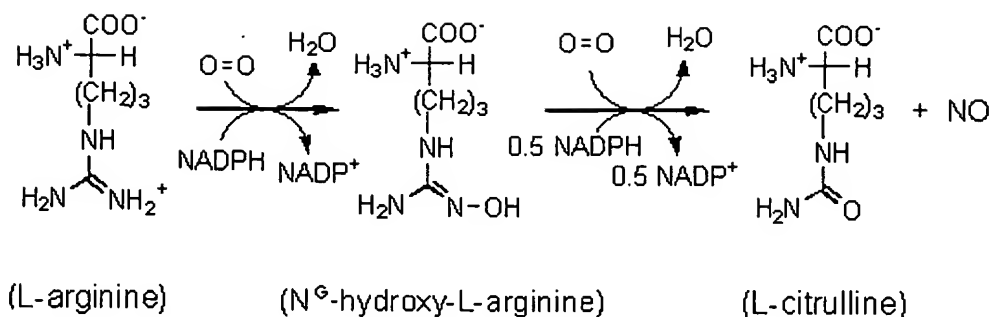
[0024] Fig.2 shows the comparative curve of the results of group I and III in the pharmacodynamic study using a compound in accordance with one embodiment of the invention.

[0025] Fig.3 shows effects of Liriodenine on eNOS protein and  $\alpha$ -tubulin expression in a rat heart that had suffered ischemia for 30 min and was then reperfused for 2-hr.

[0026] Fig.4 shows effects of Liriodenine on eNOS protein expression in human being umbilical cord vessel endothelium cells (HUVEC).

#### **Detailed Description of the Invention**

[0027] Human blood vessels can synthesize nitric oxide (NO). NO can dilate blood vessels and, therefore, NO production is closely associated with blood pressure regulation. Endogenous NO plays an important role in the vascular smooth muscle relaxation. In *ex vivo* experiments using an isolated aortic ring and local blood vessel layer and *in vivo* whole body experiments have shown that the blood vessels constrict and the blood pressures elevate upon the interruption of NO formation. In mammals, NO is synthesized from L-arginine by nitric oxide synthase (NOS). The NOS converts L-arginine to an intermediate, which is then converted to L-citrulline and NO as follows:



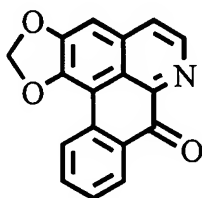
**[0028]** NOS exists in at least three isoforms, including neuronal NOS (nNOS, or type I NOS), inducible NOS (iNOS, or type II NOS), and endothelial NOS (eNOS, or type III NOS). Among these, the function of eNOS is responsible for the regulation of blood vessel tension. The function and mechanism of NO as a signal transduction messenger vary depending on where it is produced. eNOS has three major functions: (1) in nerve synapse, it produces NO as nerve in pulse conduction factor and it may contribute to brain learning and memory; (2) in blood vessel endothelia, it produces NO to relax vascular smooth muscle so as to dilate the vessel and lower blood pressure; and (3) in macrophage, it produces NO to destroy and kill tumor cells to prevent their growth.

**[0029]** nNOS and eNOS are complex enzymes, requiring calcium and calmodulin. Calcium first binds to calmodulin, then the calcium-calmodulin complex binds nNOS or eNOS to activate its catalytic activity. On the other hand, iNOS is inducible and does not depend on calcium or calmodulin. Instead, iNOS is induced by cytokines. Because iNOS is calcium-independent and calmodulin-independent, the activity of iNOS once induced cannot be easily terminated and may last for several hours, leading to overproduction of NO, which can be harmful.

**[0030]** Prior to the present invention, research on compounds that can maintain or increase eNOS activities is mostly focused on the treatment of cardiovascular diseases (e.g., arrhythmia, Su MJ, et al., Drug Development Research, 2001, 52:446-453). The present invention provides new compounds that function by a similar mechanism but can be used to prevent or treat ischemic diseases, such as stroke. The following describes methods and results of using these compounds in the treatment of ischemic diseases.

**[0031]** The invention relates to aporphine and oxoaporphine compounds for use in preventing or treating ischemic diseases. These compounds function by maintaining or

increasing endothelial nitric oxide synthase (eNOS) activities. One example of these compounds is Liriodenine, which is one of the formula VI compounds, where R1 through R5 are H. Liriodenine is used as a preferred example to demonstrate the present invention. The structure of Liriodenine, which is a plant alkaloid, is as follow:



Liriodenine

**[0032]** After Liriodenine was administrated to male Sprague Dawley rats, the effects of Liriodenine on the artery occlusive cerebral ischemia in the rats were monitored. First, permanent brain ischemia was induced by middle cerebral artery occlusion (MCAO) in the rats. At 0, 6, 24, 48, and 54 hours after the formation of middle cerebral artery occlusion (MCAO), Group I (i.e., the control group) animals were given the solvent (0.9% NaCl) (5ml/kg, i.v.); Group II animals (PT#1010853-ADD(NTU-12)(NTU-106)) were given Liriodenine (0.1mg/kg, i.v.); Group III animals were given MK-801 (0.1mg/kg, i.v.), an antagonist of the N-methyl-D-aspartic acid (NMDA) receptor. There were six rats in each group. The body temperature of each animal was measured anally before and 15 min after the administration of each treatment. Four days after the formation of MCAO, all the rats were sacrificed. Brain sections were made and stained with 2% cresol purple. The areas and volumes of cerebral ischemia lesions in each section were recorded.

**[0033]** The results are shown in Table 1, 2 and 3, for Groups I, II, and III, respectively. The comparative results among these three groups are shown in Table 4. The results may also be shown as graphs. For example, Fig. 1 shows the comparative curves of the results of group I and group II, and Fig. 2 shows the comparative curves of the results of group I and group III. From these Tables and Figures, as compared with MK-801, it is apparent that the test compound Liriodenine had more significant effects in reducing the areas and volumes of cerebral ischemia lesions in the treatment of ischemic apoplexy. The results of the body temperature measurements are shown in Tables 5, 6 and 7 for Groups I, II,

and III, respectively. Compared with the results of group I, the test compound Liriodenine did not cause significant changes in body temperatures, while MK-801 caused significant side effects, i.e. decreasing body temperatures.

**[0034]** Fig. 3 and Fig. 4 show that Liriodenine could protect eNOS or stimulate its production in ischemia-reperfusion heart and serum-deprived HUVECs (human umbilical vein endothelial cells) to achieve the aim of preventing or treating ischemic disease.

**[0035]** Fig. 3 shows the effects of Liriodenine on eNOS protein expression in a rat heart that had suffered ischemia for 30 min and then was reperfused for 2-hrs. The expression of  $\alpha$ -tubulin was used as a quantitative control. In Figure 3, lane a shows the effects on eNOS and  $\alpha$ -tubulin expression in the hearts of male rats under normal conditions. Lane b shows the effects on eNOS and  $\alpha$ -tubulin expression in the non-infarcted areas, wherein the left anterior descending branch coronary artery (LAD) of the heart had been ligated and then reperfused with a vehicle-containing solution. Lane c shows the effect on eNOS and  $\alpha$ -tubulin expression in infarcted areas, wherein the left anterior descending branch coronary artery of the heart had been ligated and then reperfused with a vehicle-containing solution. Lane d shows the effects on eNOS and  $\alpha$ -tubulin expression in non-infarcted areas, wherein the heart was pretreated with 1  $\mu$ M Liriodenine and then the left anterior descending branch coronary artery of the heart was ligated and then reperfused with 1 $\mu$ M Liriodenine. Lane e shows the effects on eNOS and  $\alpha$ -tubulin expression in infarcted areas, wherein the heart was pretreated with 1  $\mu$ M Liriodenine and then the left anterior descending branch coronary artery of the heart was ligated and then reperfused with 1 $\mu$ M Liriodenine. From the difference in expression between eNOS and  $\alpha$ -tubulin, it is clear that the expression of eNOS in the heart can approach the normal value after treatment with Liriodenine solution. These results show that a compound of the present invention (such as Liriodenine) can improve the expression of eNOS or maintain its expression at a constant level.

**[0036]** Fig. 4 shows the effects of Liriodenine on eNOS protein expression in human umbilical vein endothelium cells (HUVEC). As shown in this figure, eNOS protein level was significantly reduced under serum-deprived (SD) condition for 24 hours. Administering Liriodenine from 0.1 to 1  $\mu$ M can increase eNOS protein levels in serum-

deprived HUVECs in a dose-dependent manner. The levels of eNOS protein expression are quantified using densitometer software, relative to the level of eNOS protein expression under the SD condition, which is set as 1 in the histogram. The results shown in the Figure 4 are representative of three independent tests. Asterisks in the histogram indicate that the results have  $P < 0.05$ , meaning the difference is significant from the level of HUVEC eNOS under SD. From the results in Fig. 4, it is clear that expression levels of eNOS increase significantly after the HUVEC was treated with 0.3 and 1  $\mu$ M of Liriodenine. This result shows that Liriodenine is capable of maintaining eNOS expression level or enhancing the formation of eNOS.

**[0037]** Therefore, a composition of the invention can maintain the endothelial function by increasing eNOS activity. The functions of eNOS include inhibiting leukocyte and platelet adhesion, as well as regulating blood flow to organs etc. The functions of various forms of NOS on the cerebral ischemia are confirmed in a transgenic animal model. In local cerebral ischemia, the infarction is more severe in the eNOS gene-knockout mice than in the wild-type mice. In contrast, iNOS knockout mice or nNOS knockout mice have less infarction in local cerebral ischemia than the wild-type mice.

**[0038]** Results from the above test show that the compounds of the invention can decrease the volume and area of cerebral infarct in rats without the side effects caused by MK801 (an NMDA receptor antagonist).

**[0039]** In addition, the compound described above can treat ischemic diseases not only in rats, but also in the mammals and human being. The ischemic diseases include ischemic cerebral thrombosis, ischemic cerebral embolism, hypoxic ischemic encephalopathy, ischemic cardiac disease or ischemic enteropathy, as well as ischemic cerebral apoplexy.

<b>Table 1 (Summary)</b>					
Calculated Decrease in Total Volume of Ischemic Lesions in the Brains of MCAO Rats by the Test Compound					
Treatment	Route	Dose	N	Infarcted Volume ( $\bar{X} \pm \text{SEM mm}^3$ )	% Decrease. ( $\bar{X} \pm \text{SEM}$ )
Vehicle Control	i.v.	5ml/kg $\times$ 6	6	109.92 $\pm$ 2.96	-
PT#1010853-ADD (NTU-12) (NTU-106)	i.v.	0.1mg/kg $\times$ 6	6	71.37 $\pm$ 11.87*	35.07 $\pm$ 10.80
MK-801	i.v.	0.1mg/kg $\times$ 6	6	75.17 $\pm$ 13.74*	31.61 $\pm$ 12.50

\*P<0.05

Table 2							
Area of Ischemic Lesions in Vehicle Control (0.9% NaCl, 5 ml/kg×6, i.v.) MCAO Rats							
Slice#	Infarcted Area (mm <sup>2</sup> ) of Each Test Animal						X±SEM
	1	2	3	4	5	6	N=6
BW(g)	250	240	240	250	260	270	
1	1.52	0	0	0	1.20	0	0.45±0.29
2	5.05	0	0	0	4.20	0	1.54±0.98
3	8.50	1.18	3.16	0	5.11	4.57	3.75±1.24
4	10.80	4.50	4.62	2.68	8.25	7.17	6.34±1.21
5	12.60	8.71	6.25	4.44	9.45	9.28	8.45±1.15
6	15.00	9.38	8.68	6.49	13.00	9.97	10.42±1.26
7	15.90	11.00	9.67	9.23	15.30	12.30	12.23±1.15
8	16.00	12.00	10.80	10.30	16.90	13.00	13.17±1.11
9	17.00	12.70	11.50	12.20	17.90	13.10	14.07±1.10
10	17.60	14.20	11.60	15.30	17.60	15.30	15.27±0.92
11	16.60	15.60	12.70	15.70	16.60	15.60	15.47±0.59
12	15.80	13.70	12.80	15.80	15.90	17.70	15.28±0.72
13	13.50	13.60	13.00	16.80	15.70	18.10	15.12±0.85
14	13.30	12.60	12.70	14.80	13.40	14.70	13.58±0.39
15	11.60	11.70	11.70	14.40	13.30	14.70	12.90±0.58
16	11.50	11.10	11.50	14.20	12.70	13.90	12.48±0.54
17	11.20	11.00	11.30	14.10	12.20	10.80	11.77±0.51
18	10.00	10.90	11.20	11.80	10.70	10.80	10.90±0.24
19	9.97	10.80	11.10	11.50	10.50	10.40	10.71±0.22
20	9.96	10.70	11.00	11.00	9.82	9.90	10.40±0.23
21	9.70	10.60	10.30	11.00	8.73	9.80	10.02±0.33
22	9.60	9.72	9.76	10.50	8.68	8.97	9.54±0.26
23	8.77	8.80	9.50	9.60	7.47	8.75	8.81±0.31
24	8.74	8.44	8.74	8.86	6.30	8.35	8.24±0.40
25	8.38	8.31	7.83	8.60	3.95	7.10	7.36±0.72
26	7.96	7.73	7.12	8.38	3.82	6.55	6.93±0.67
27	5.81	7.70	6.82	6.45	1.59	6.06	5.74±0.87
28	5.73	7.41	6.62	5.87	0	4.96	5.10±1.07
29	5.00	6.71	4.95	4.00	0	0.94	3.60±1.06
30	3.09	2.61	3.83	3.11	0	0.58	2.20±0.63
Total mm <sup>2</sup>	316.18	273.40	260.75	277.11	280.27	283.35	281.84±7.58
Total mm <sup>3</sup>	123.31	106.63	101.69	108.07	109.31	110.51	109.92±2.96

Table 3							
Area of Ischemic Lesions in PT#1010853-ADD (NTU-12) (NTU-106) (0.1mg/kg×6, i.v.) MCAO Rats							
Slice#	Infarcted Area (mm <sup>2</sup> ) of Each Test Animal						X±SEM
	1	2	3	4	5	6	N=6
BW(g)	230	240	240	250	240	230	
1	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0
5	0	3.86	2.61	0	0	1.97	1.41±0.68
6	0	6.38	4.31	0	0	2.95	2.27±1.11
7	0.93	8.47	5.33	0	0	4.30	3.17±1.40
8	3.20	8.55	6.94	0	0	8.17	4.48±1.61
9	4.52	9.10	7.91	0	0.61	7.68	4.97±1.60
10	4.73	9.11	9.92	0	2.44	7.10	5.55±1.58
11	8.54	10.30	10.50	1.75	6.97	7.09	7.53±1.31
12	9.95	12.60	10.90	2.80	10.70	6.22	8.86±1.49
13	10.60	12.00	13.80	3.44	11.30	5.23	9.39±1.67
14	10.70	11.20	10.71	3.56	11.40	4.93	8.75±1.44
15	11.50	10.70	9.99	3.84	12.54	3.67	8.71±1.60
16	12.10	10.60	9.83	5.92	14.30	2.48	9.20±1.76
17	12.40	10.50	9.70	6.59	14.50	1.17	9.14±1.93
18	13.30	10.40	9.68	7.33	15.10	0	9.30±2.17
19	13.90	10.40	9.55	7.37	15.70	0	9.49±2.26
20	14.80	9.91	9.10	9.30	14.60	0	9.62±2.20
21	15.20	9.31	9.08	8.56	14.29	0	9.41±2.21
22	14.20	9.14	8.92	8.13	12.50	0	8.82±2.01
23	13.90	9.01	8.85	7.79	11.44	0	8.50±1.92
24	12.10	7.90	6.89	7.45	11.10	0	7.57±1.74
25	10.50	7.86	6.71	6.76	11.00	0	7.14±1.61
26	10.40	7.80	6.50	6.23	10.70	0	6.94±1.59
27	10.30	7.68	6.05	6.16	10.60	0	6.80±1.58
28	9.74	6.98	5.48	6.08	9.27	0	6.26±1.43
29	8.03	5.88	4.80	5.35	8.79	0	5.47±1.27
30	7.94	4.30	4.40	3.95	4.98	0	4.26±1.04
Total mm <sup>2</sup>	243.48	229.94	208.46	118.36	234.83	62.96	183.00±30.43
Total mm <sup>3</sup>	94.96	89.68	81.30	46.16	91.58	24.55	71.37±11.87*
%Inh	13.61	18.41	26.04	58.01	16.68	77.67	35.07±10.80



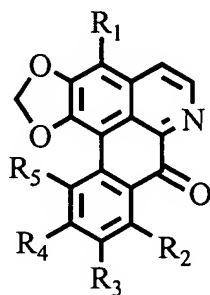
Table 4							
Area of Ischemic Lesions in MK-801(0.1mg/kg×6,i.v.) MCAO Rats							
Slice#	Infarcted Area (mm <sup>2</sup> ) of Each Test Animal						X±SEM
	1	2	3	4	5	6	N=6
BW(g)	220	250	250	260	240	270	
1	0	0	0	0	0	0	0
2	0	1.91	0	0	0	0	0.32±0.32
3	0	3.53	0	0	0	0.97	0.75±0.58
4	0	5.86	0	0	0	3.76	1.60±1.05
5	1.99	6.74	0	0	2.52	5.58	2.81±1.15
6	4.33	8.02	0	0	4.32	7.78	4.08±1.44
7	5.86	10.50	2.74	0	7.54	11.10	6.29±1.78
8	6.97	11.90	5.11	1.18	9.83	11.70	7.78±1.71
9	8.63	12.30	6.39	2.98	13.90	12.00	9.37±1.70
10	10.60	12.80	7.72	4.32	12.20	12.40	10.01±1.37
11	12.80	14.00	8.21	5.78	12.00	11.70	10.75±1.27
12	12.90	12.20	8.81	5.24	10.70	10.20	10.01±1.12
13	13.90	10.80	8.93	4.73	10.10	10.10	9.76±1.22
14	15.00	10.80	10.10	4.04	10.10	9.94	10.00±1.43
15	12.80	10.70	9.59	2.64	10.00	9.74	9.24±1.41
16	12.80	10.20	8.70	0	9.84	8.83	8.40±1.78
17	12.70	9.82	8.20	0	9.66	8.82	8.20±1.76
18	12.00	9.65	8.11	0	9.10	8.81	7.95±1.68
19	11.90	9.42	7.78	0	9.05	8.68	7.81±1.66
20	11.30	9.35	7.74	0	9.01	8.03	7.57±1.60
21	10.70	9.08	7.66	0	8.67	8.00	7.35±1.53
22	10.10	9.04	7.31	0	8.60	7.92	7.16±1.48
23	10.10	8.81	7.22	0	8.45	7.76	7.06±1.47
24	9.65	8.68	6.80	0	8.16	7.72	6.84±1.42
25	9.64	8.44	6.65	0	8.14	7.30	6.69±1.40
26	8.88	8.01	5.95	0	7.66	6.87	6.23±1.31
27	8.45	7.77	3.80	0	7.33	6.40	5.63±1.30
28	8.44	7.68	3.47	0	7.17	6.00	5.46±1.30
29	7.98	5.38	3.09	0	5.31	5.31	4.51±1.10
30	3.89	5.02	3.03	0	4.08	2.94	3.16±0.70
Total mm <sup>2</sup>	254.31	258.41	163.11	30.91	223.44	226.36	192.76±35.23
Total mm <sup>3</sup>	99.18	100.78	63.61	12.05	87.14	88.28	75.17±13.74*
%Inh.	9.77	8.32	42.13	89.04	20.72	19.69	31.61±12.50

Table 5								
Body Temperature (°C) At 0 Minute Before (Pre) And 15 Min After (Post) Compound Treatment								
Treatment	Route	N	0 Hour Dosage			6 Hours Dosage		
			Pre	Post	$\Delta$	Pre	Post	$\Delta$
Vehicle (5ml/kg)	IV	1	37.9	36.0	-1.9	37.4	37.9	0.5
	IV	2	38.1	36.8	-1.3	37.6	38.4	0.8
	IV	3	37.9	36.5	-1.4	37.3	38.0	0.7
	IV	4	38.4	37.8	-0.6	38.1	36.4	-1.7
	IV	5	38.1	38.7	0.6	37.3	38.0	0.7
	IV	6	37.5	36.1	-1.4	37.9	37.8	-0.1
	X		38.0	37.0	-1.0	37.6	37.8	0.1
	SEM		0.1	0.4	0.4	0.1	0.3	0.4
Treatment	Route	N	24 Hours Dosage			30 Hours Dosage		
			Pre	Post	$\Delta$	Pre	Post	$\Delta$
Vehicle (5ml/kg)	IV	1	38.3	38.0	-0.3	37.7	37.2	-0.5
	IV	2	38.1	37.9	-0.2	37.7	38.2	0.5
	IV	3	37.6	36.2	-1.4	37.9	37.5	-0.4
	IV	4	38.2	37.5	-0.7	37.3	37.9	0.6
	IV	5	37.9	37.3	-0.6	38.1	37.8	-0.3
	IV	6	38.0	37.9	-0.1	37.6	38.0	0.4
	X		38.0	37.5	-0.5	37.7	37.8	0.1
	SEM		0.1	0.3	0.2	0.1	0.1	0.2
Treatment	Route	N	48 Hours Dosage			54 Hours Dosage		
			Pre	Post	$\Delta$	Pre	Post	$\Delta$
Vehicle (5ml/kg)	IV	1	37.9	37.0	-0.9	37.9	38.0	0.1
	IV	2	38.0	37.5	-0.5	37.6	37.9	0.3
	IV	3	37.4	38.0	0.6	38.2	38.1	-0.1
	IV	4	37.3	38.5	1.2	38.7	39.0	0.3
	IV	5	37.1	37.7	0.6	38.5	38.3	-0.2
	IV	6	37.6	37.5	-0.1	37.7	38.0	0.3
	X		37.6	37.7	0.2	38.1	38.2	0.1
	SEM		0.1	0.2	0.3	0.2	0.2	0.1

Table 6								
Body Temperature (°C) At 0 Minute Before (Pre) And 15 Min After(Post) Compound Treatment								
Treatment	Route	N	0 Hour Dosage			6 Hours Dosage		
			Pre	Post	△	Pre	Post	△
PT#1010853-ADD (NTU-12) (NTU-106) (0.1mg/kg×6)	IV	1	37.0	36.3	-0.7	37.8	38.3	0.5
	IV	2	38.0	37.1	-0.9	37.9	38.5	0.6
	IV	3	37.0	37.6	0.6	38.3	38.3	0
	IV	4	36.9	37.9	1.0	38.2	38.6	0.4
	IV	5	37.4	37.1	-0.3	38.1	37.8	-0.3
	IV	6	37.5	36.9	-0.6	37.5	38.4	0.9
	X		37.3	37.1	-0.1	38.0	38.3	0.3
	SEM		0.2	0.2	0.3	0.1	0.1	0.2
Treatment	Route	N	24 Hours Dosage			30 Hours Dosage		
			Pre	Post	△	Pre	Post	△
PT#1010853-ADD (NTU-12) (NTU-106) (0.1mg/kg×6)	IV	1	37.6	36.8	-0.8	38.5	37.9	-0.6
	IV	2	38.6	38.2	-0.6	37.7	37.7	0
	IV	3	38.1	37.9	-0.2	37.9	38.3	0.4
	IV	4	38.0	37.8	-0.2	38.9	38.4	-0.5
	IV	5	38.0	37.7	-0.3	38.3	38.0	-0.3
	IV	6	37.8	38.0	0.2	38.0	37.9	-0.1
	X		38.0	37.7	-0.3	38.2	38.1	-0.2
	SEM		0.1	0.2	0.1	0.2	0.1	0.1
Treatment	Route	N	48 Hours Dosage			54 Hours Dosage		
			Pre	Post	△	Pre	Post	△
PT#1010853-ADD (NTU-12) (NTU-106) (0.1mg/kg×6)	IV	1	37.9	37.5	-0.4	37.9	38.2	0.3
	IV	2	38.2	38.0	-0.2	37.5	38.0	0.5
	IV	3	38.2	37.6	-0.6	38.0	37.6	-0.4
	IV	4	38.5	38.1	-0.4	38.0	38.2	0.2
	IV	5	37.7	38.0	0.3	37.4	37.8	0.4
	IV	6	38.2	38.0	-0.2	37.7	38.0	0.3
	X		38.1	37.9	-0.3	37.7	38.0	0.2
	SEM		0.1	0.1	0.1	0.1	0.1	0.1

Table 7								
Body Temperature (°C) At 0 Minute Before (Pre) And 15 Min After(Post) Compound Treatment								
Treatment	Route	N	0 Hour Dosage			6 Hours Dosage		
			Pre	Post	△	Pre	Post	△
MK-801 (0.1mg/kg×6)	IV	1	39.0	36.5	-2.5	38.1	38.6	0.5
	IV	2	38.3	35.9	-2.4	37.8	37.5	-0.3
	IV	3	37.9	35.4	-2.5	37.7	37.7	0
	IV	4	38.7	36.1	-2.6	37.8	37.7	-0.1
	IV	5	36.8	36.0	-0.8	37.7	37.3	-0.4
	IV	6	37.1	35.6	-1.5	38.0	38.7	0.7
	X		38.0	35.9	-2.0	37.8	37.9	0.1
	SEM		0.4	0.2	0.3	0.1	0.2	0.2
Treatment	Route	N	24 Hours Dosage			30 Hours Dosage		
			Pre	Post	△	Pre	Post	△
MK-801 (0.1mg/kg×6)	IV	1	37.7	38.2	0.5	37.0	37.2	0.2
	IV	2	37.5	37.2	0.3	37.7	38.0	0.3
	IV	3	37.6	37.5	-0.1	37.3	38.2	0.9
	IV	4	37.8	38.0	0.2	37.9	37.9	0
	IV	5	37.9	37.3	-0.6	38.1	37.5	-0.6
	IV	6	37.3	36.9	-0.4	37.4	38.2	0.8
	X		37.8	37.5	0	37.6	37.8	0.3
	SEM		0.1	0.2	0.2	0.2	0.2	0.2
Treatment	Route	N	48 Hours Dosage			54 Hours Dosage		
			Pre	Post	△	Pre	Post	△
MK-801 (0.1mg/kg×6)	IV	1	38.0	37.3	-0.7	37.8	38.5	0.7
	IV	2	38.0	37.3	-0.7	37.5	38.1	0.6
	IV	3	37.5	37.2	-0.3	37.4	38.0	0.6
	IV	4	37.7	38.4	0.7	37.9	39.0	1.1
	IV	5	37.7	38.0	0.3	37.7	37.6	-0.1
	IV	6	37.3	36.9	-0.4	37.7	36.8	-0.9
	X		37.7	37.5	-0.2	37.7	38.0	0.3
	SEM		0.1	0.2	0.2	0.1	0.3	0.3

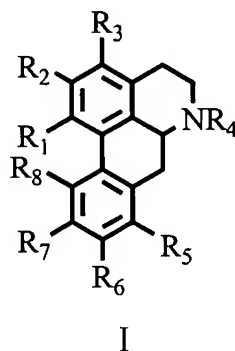
[0040] In above-mentioned specific formula VI compound (Liriodenine) for preventing or treating ischemic disease by maintaining or increasing eNOS, R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>, R<sub>4</sub> and R<sub>5</sub> are H.



VI

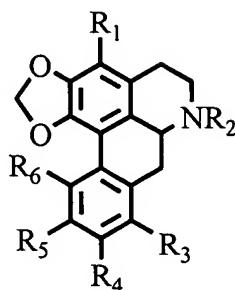
**[0041]** In addition to Liriodenine, other aporphine and oxoaporphine compounds having the same activity may include formula VI compounds, where  $R_1$  and  $R_2$  are each selected from OH, O-acyl, OMe, F, Cl, Br,  $NO_2$  and CN;  $R_3$  and  $R_4$  are each selected from OH, OEt,  $O^nPr$ ,  $O^iPr$  and O-acyl; and  $R_5$  is OH, O-acyl, or OMe. All these compounds can be used to prevent or treat ischemic diseases in human or other mammals.

**[0042]** Besides the above-mentioned formula VI aporphine and oxoaporphine compounds, other aporphine and oxoaporphine compounds for preventing or treating ischemic diseases in accordance with some embodiments of the invention may include formula I compounds:



where  $R_1$ ,  $R_2$ ,  $R_6$ , and  $R_7$  are each selected from H, OH, O-acyl, OMe, OEt,  $O^nPr$  and  $O^iPr$ ;  $R_3$  and  $R_5$  are each selected from H, OH, O-acyl, OMe, F, Cl, Br,  $NH_2$ ,  $NO_2$  and CN;  $R_4$  is allyl or  $C_nH_{2n+1}$ ,  $n \geq 0$ ; and  $R_8$  is H, OH, or OMe.

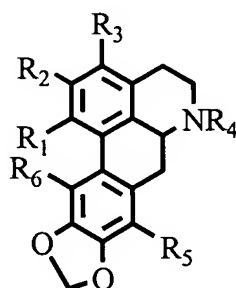
**[0043]** The aporphine and oxoaporphine compounds for preventing or treating ischemic diseases in accordance with some embodiments of the invention may include formula II compounds:



## II

where  $R_1$  and  $R_3$  are each selected from H, OH, O-acyl, OMe, F, Cl, Br,  $NH_2$ ,  $NO_2$  and CN;  $R_2$  is allyl or  $C_nH_{2n+1}$ ,  $n \geq 0$ ,  $R_4$ ; and  $R_5$  is selected from H, OH, O-acyl, OMe, OEt,  $O^nPr$ , and  $O^iPr$ ; and  $R_6$  is H, OH, O-acyl, or OMe.

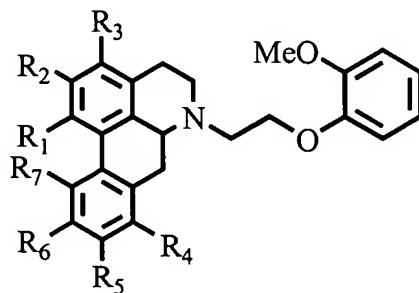
**[0044]** The aporphine and oxoaporphine compounds for preventing or treating ischemic diseases in accordance with some embodiments of the invention may include formula III compounds:



III

where  $R_1$  and  $R_2$  are each selected from H, OH, O-acyl, OMe, OEt,  $O^nPr$  and  $O^iPr$ ;  $R_3$  and  $R_5$  are each selected from H, OH, O-acyl, OMe, Cl, Br,  $NH_2$ ,  $NO_2$  and CN;  $R_4$  is allyl or  $C_nH_{2n+1}$ ,  $n \geq 0$ ; and  $R_6$  is H, OH, O-acyl, or OMe.

**[0045]** The aporphine and oxoaporphine compounds for preventing or treating ischemic diseases in accordance with some embodiments of the invention may include formula IV compounds:

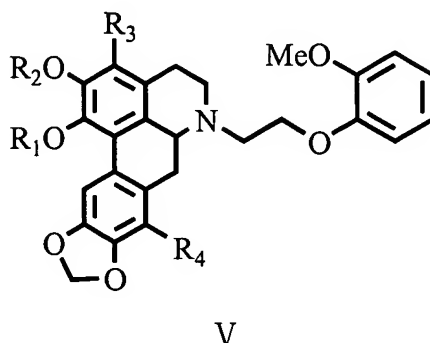


IV

where  $R_1$ ,  $R_2$ ,  $R_5$  and  $R_6$  are each selected from H, OH, O-acyl, OMe, OEt,  $O^nPr$ , and  $O^iPr$ ;  $R_3$  and  $R_4$  are each selected from H, OH, O-acyl, OMe, F, Cl, Br,  $NH_2$ ,  $NO_2$  and CN; and

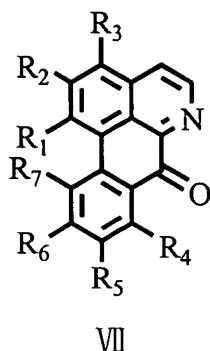
R<sub>7</sub> is H, OH, O-acyl, or OMe.

**[0046]** The aporphine and oxoaporphine compounds for preventing or treating ischemic diseases in accordance with some embodiments of the invention may include formula V compounds:



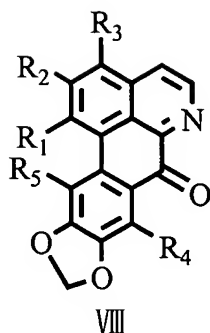
where R<sub>1</sub> and R<sub>2</sub> are each selected from H, acyl, Me, Et, <sup>n</sup>Pr and <sup>i</sup>Pr; R<sub>3</sub> and R<sub>4</sub> are each selected from H, OH, O-acyl, OMe, F, Cl, Br, NH<sub>2</sub>, NO<sub>2</sub> and CN.

**[0047]** The aporphine and oxoaporphine compounds for preventing or treating ischemic diseases in accordance with some embodiments of the invention may include formula VII compounds:



where R<sub>1</sub>, R<sub>2</sub>, R<sub>5</sub> and R<sub>6</sub> are each selected from H, OH, OAc, OMe, OEt, O<sup>n</sup>Pr and O<sup>i</sup>Pr; R<sub>3</sub> and R<sub>4</sub> are each selected from H, OH, O-acyl, OMe, F, Cl, Br, NO<sub>2</sub> and CN; R<sub>7</sub> is H, O-acyl, or OMe.

**[0048]** The aporphine and oxoaporphine compounds for preventing or treating ischemic diseases in accordance with some embodiments of the invention may include formula VIII compounds:



where  $R_1$  and  $R_2$  are each selected from H, OH, O-acyl, OMe, OEt,  $O^nPr$  and  $O^iPr$ ;  $R_3$  and  $R_4$  are each selected from H, OH, O-acyl, OMe, F, Cl, Br,  $NO_2$  and CN; and  $R_5$  is H, OH, O-acyl, or OMe.

**[0049]** Any of the above-mentioned formula I to VIII compounds can be used in combination or with a pharmaceutical acceptable carrier or excipient. Any carrier or excipient known in the art (e.g., lactose) may be used with embodiments of the invention.

**[0050]** All the aporphine and oxoaporphine compounds with different structures described above can be used to treat ischemic diseases in mammal or human being, and the ischemic diseases include ischemic cerebra apoplexy, ischemic cerebral thrombosis, ischemic cerebral embolism, hypoxic ischemic encephlopathy, ischemic cardiac disease, and ischemic enteropathy.

**[0051]** The above describes various aporphine and oxoaporphine compounds and their treatment effects. The following examples describe the preparations of the aporphine and oxoaporphine compounds in accordance with embodiments of the invention.

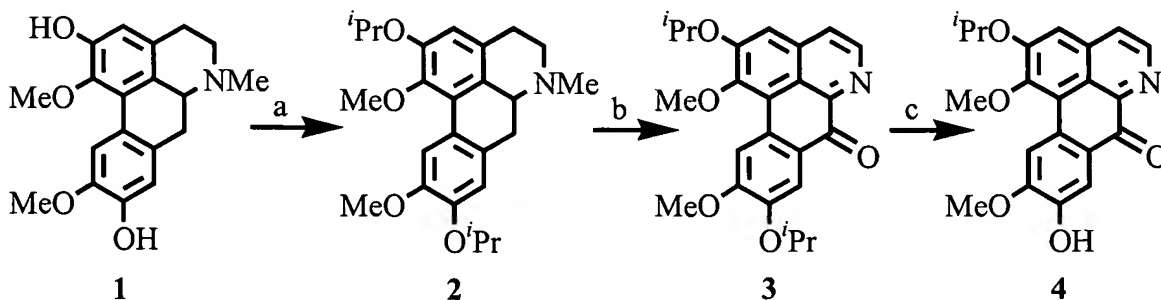
### Example 1

**[0052]** Preparation of 2,9-Diisopropoxy-1,10-dimethoxy-7-oxoaporphine(3), the formula VII compound, wherein  $R_1=R_6=OMe$ ,  $R_2=R_5=OiPr$ ,  $R_3=R_4=R_7=H$ ; and 9-Hydroxy-2-isopropoxy-1,10-dimethoxy-7-oxoaporphine(4), the formula VII compound, wherein  $R_1=R_6=OMe$ ,  $R_2=OiPr$ ,  $R_3=R_4=R_7=H$ ,  $R_5=OH$ .



**[0053]** 1. Preparation of 2,9-Diisopropoxy-1,10-dimethoxy-N-methylaporphine (2), the formula I compound, wherein  $R_1 = R_7 = \text{OMe}$ ,  $R_2 = R_6 = \text{O}^i\text{Pr}$ ,  $R_3 = R_5 = R_8 = \text{H}$ ,  $R_4 = \text{Me}$ .

**[0054]** The mixture of boldine (1) (1.63g, 5mmol), anhydrous alcohol (50ml), and anhydrous potassium carbonate (3.0g) in a 250 ml round bottom flask was stirred in an oil bath (70°C). A solution of 2-iodopropane (3.4g, 20 mmol) in anhydrous alcohol solution (10ml) was added dropwise over 1 hour. The reaction was allowed to proceed for 8 hours and then the solution was cooled to room temperature. The inorganic sediments were filtered and washed with alcohol. The combined filtrate and washings were concentrated under reduced pressure. The residue obtained was dissolved in chloroform (150ml), then extracted successively with 10% sodium hydroxide solution (50ml) and water (50ml x3) to remove the impurity. The chloroform layer is dried with anhydrous sodium sulfate and concentrated under reduced pressure to give a residue, which was purified on a basic alumina column, and eluted with chloroform to obtain 2,9-diisopropoxy-1,10-dimethoxy-N-methylaporphine (2) (1.54g, 75% yield):  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ )  $\delta$  1.40 (6H, d,  $J=6.1$  Hz, 2x  $\text{CH}_3$ ), 1.43 (6H, d,  $J=6.2$  Hz, 2x  $\text{CH}_3$ ), 2.52 (3H, s,  $\text{NCH}_3$ ), 3.64 (3H, s, 1- $\text{OCH}_3$ ), 3.85 (3H, s, 10- $\text{OCH}_3$ ), 4.54 (1H, m) and 4.59 (1H, m) (2x  $\text{OCH}$ ), 6.56 (1H, s, H-3), 6.76 (1H, s, H-8), 8.06 (1H, s, H-11).



a.  $^i\text{PrI}/\text{K}_2\text{CO}_3$ , abs. EtOH, 70 °C, 8hr, 75%; b.  $\text{Pb}(\text{OAc})_4$ , HOAc, rt, 12 hr, 50%; c. 4%  $\text{H}_2\text{SO}_4$ -HOAc,  $\text{N}_2$ ,  $\Delta$ , 1 hr, 22%.

**[0055]** 2. Preparation of 2,9-diisopropoxy-1,10-dimethoxy-7-oxoaporphine (3), a formula VII compound, wherein  $R_1=R_6=\text{OMe}$ ,  $R_2=R_5=\text{O}^i\text{Pr}$ ,  $R_3=R_4=R_7=\text{H}$ .

**[0056]** To a solution of compound (2) (137mg, 330 $\mu$ M) in acetic acid (5ml) was added lead tetraacetate (95%, 483mg, 1.09mmol). The reaction mixture was stirred for 12 hours at room temperature, then water (150ml) was added, followed by successive extraction with chloroform (50ml $\times$ 4). The combined chloroform layers were washed successively with saturated sodium bicarbonate aqueous solution (50ml), 10% sodium hyposulfite aqueous solution (50ml) and water (50ml $\times$ 2), dried with anhydrous sodium sulfate, and concentrated under reduced pressure. The residue obtained was purified on a silica gel column and eluted with chloroform to give 2,9-diisopropoxy-1,10-dimethoxy-7-oxoaporphine (3) (68mg, 50% yield): m.p. 82-84 $^{\circ}$ C; IR(KBr) $\nu_{\max}$  2976, 2933, 1654, 1590, 1563, 1508, 1459, 1431, 1416, 1359, 1296, 1275, 1241, 1216, 1138, 1110, 1057, 1009, 995, 925, 887, 864, 782  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR(200 MHz,  $\text{CDCl}_3$ )  $\delta$  1.44 (6H, d,  $J=6.1$  Hz, 2x  $\text{CH}_3$ ), 1.53 (6H, d,  $J=6.1$  Hz, 2x  $\text{CH}_3$ ), 4.01 (3H, s, 1- $\text{OCH}_3$ ), 3.85 (3H, s, 10- $\text{OCH}_3$ ), 4.87 (2H, m) (2x OCH), 7.79 (1H, d,  $J=5.4$  Hz, H-4), 7.96 (1H, s, H-8), 8.75 (1H, s, H-11), 8.86 (1H, d,  $J=5.4$  Hz, H-5);  $^{13}\text{C}$  NMR(50 MHz,  $\text{CDCl}_3$ )  $\delta$  21.7 (2C, q), 21.9 (2C, q), 56.0 (q), 60.4 (q), 70.9 (d), 71.2 (d), 107.2 (d), 110.6 (d), 112.2 (d), 120.2 (s), 121.3 (s), 123.2 (d), 126.7 (s), 128.8 (s), 135.4 (s), 145.9 (d), 145.3 (s), 147.8 (s), 151.5 (s), 154.6 (s), 154.8 (s), 181.3 (s); ESI MS (positive):  $[\text{M}+\text{H}]^+$  408.

**[0057]** 3: Preparation of 9-hydroxy-2-isopropoxy-1,10-dimethoxy-7-oxoaporphine (4), a formula II compound, wherein  $\text{R}_1 = \text{R}_6 = \text{OMe}$ ,  $\text{R}_2 = \text{R}_5 = \text{O}^i\text{Pr}$ ,  $\text{R}_3 = \text{R}_4 = \text{R}_7 = \text{H}$ .

**[0058]** The solution of compound (3) (50mg) in acetic acid-sulfuric acid solution (96:4, 5ml) was refluxed for 1 hour under nitrogen. After cooling to room temperature, the solution was evaporated under vacuum. The residue was neutralized with ammonia water and extracted with chloroform (100ml  $\times$ 2). The combined chloroform layers were washed with water (50ml $\times$ 2), dried over anhydrous sodium sulfate, concentrated under reduced pressure. The residue obtained was purified on a silica gel column and eluted with chloroform-methanol (99 : 3) to obtain a brown solid, 9-hydroxy-2-isopropoxy-1,10-dimethoxy-7-oxoaporphine (4) (10mg, 22% yield): m.p. 240-242 $^{\circ}$ C; IR(KBr)  $\nu_{\max}$  3422, 3008, 2977, 2932, 1728, 1651, 1593, 1513, 1461, 1417, 1380, 1351, 1280, 1247,

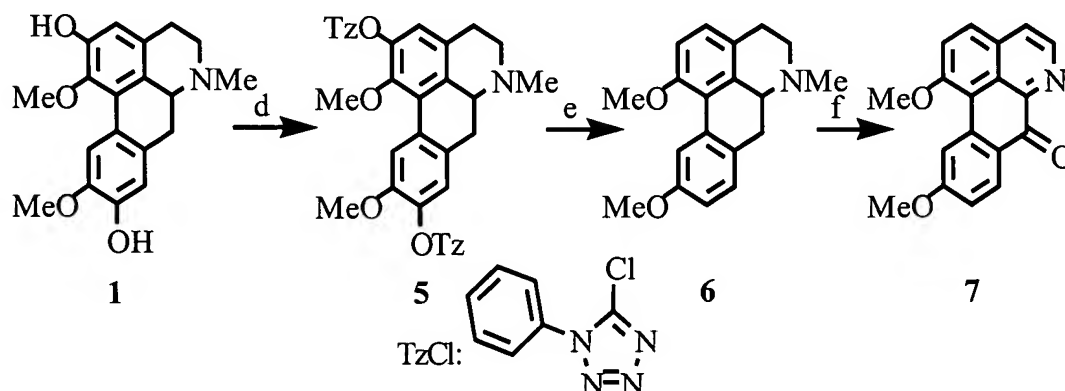
1213, 1149, 1116, 1059, 1013, 932, 894, 866, 822  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (200 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  1.51 (6H, d,  $J=6.0$  Hz, 2x  $\text{CH}_3$ ), 4.01 (3H, s,  $\text{OCH}_3$ ), 4.02 (3H, s,  $\text{OCH}_3$ ), 4.87 (1H, m, OCH), 7.25 (1H, s, H-3), 7.74 (1H, s, H-8), 7.83 (1H, d,  $J=4.8$  Hz, H-4), 8.63 (1H, d,  $J=4.8$  Hz, H-5), 8.68 (1H, s, H-11); ESI MS (positive):  $[\text{M}+\text{H}]^+$  366.

### Example 2

**[0059]** Preparation of 1,10-dimethoxy-7-oxoaporphine (7), a formula VII compound, wherein  $\text{R}_1 = \text{R}_6 = \text{Me}$ ,  $\text{R}_2 = \text{R}_3 = \text{R}_4 = \text{R}_5 = \text{R}_7 = \text{H}$ .

**[0060]** 1. Preparation of 1,10-dimethoxy-N-methylaporphine (6), a formula I compound, wherein  $\text{R}_1 = \text{R}_7 = \text{OMe}$ ,  $\text{R}_2 = \text{R}_3 = \text{R}_5 = \text{R}_6 = \text{R}_8 = \text{H}$ ,  $\text{R}_4 = \text{Me}$ .

**[0061]** Boldine [(1), 10.0 g, 30.58 mmol], acetonitrile (350 ml), anhydrous potassium carbonate (8.4 g, 61 mmol) and 5-chloro-1-phenyltetrazole (TzCl, 12.14 g, 33.64 mmol) are placed in a 500-ml round bottom flask in sequence. The mixture was heated under reflux for 24 hours. After cooling to room temperature, the insoluble inorganic salts were removed by filtration, and the sediment was washed with acetonitrile. The filtrate and acetonitrile washings were concentrated under reduced pressure to give a residue, which was suspended in chloroform (400ml) and then extracted with water (200 ml x 2) to remove the impurity. The chloroform layer, after being dried with anhydrous sodium sulfate, was concentrated. The residue was purified on a silica gel column (500g) and eluted with chloroform-methanol (99 : 1) to give 2,9-O-bis(1-phenyltetrazol-5-yl)-1,10-dimethoxy-N-methylaporphine (5) (18g, 96% yield):  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  2.55 (3H, s,  $\text{NCH}_3$ ), 3.46 (3H, s, 1- $\text{OCH}_3$ ), 3.76 (3H, s, 10- $\text{OCH}_3$ ), 7.19 (1H, s, H-3), 7.31 (1H, s, H-8), 7.42-7.60 (6H, m), and 7.83-7.87 (4H, m) ( $\text{C}_6\text{H}_5 \times 2$ ), 8.04 (1H, s, H-11).



d.  $\text{TzCl/K}_2\text{CO}_3$ , MeCN,  $\Delta$ , 24 hrs, 96%; e. 10% Pd-C,  $\text{H}_2$  (120 psi), HOAc, 50 °C, 3 d, 90%; f.  $\text{Ti}(\text{OAc})_3$ , HOAc, 70 °C, 1 hr, 69%.

**[0062]** To a solution of compound (5) (8g) in acetic acid (55 ml) was added palladium carbon (10%, 1g). The suspension was catalytically hydrogenated ( $\text{H}_2$ , 120psi) at 50°C for 3 days. After cooling, the reaction mixture was filtered through a Celite pad and the sediment was washed with chloroform. The combined filtrate and washing were concentrated under reduced pressure. The residue obtained was dissolved in chloroform (200ml), extracted with 10% sodium hydroxide aqueous solution (50ml $\times$ 2) and water (100 $\times$ 2) to remove impurities. The chloroform layer, after being dried with anhydrous sodium sulfate, was concentrated under reduced pressure to give a residue, which was purified on a silica gel column and eluted with chloroform-methanol (98 : 2) to give 1,10-dimethoxy-N-methylaporphine (6) (4.12g, 90% yield):  $^1\text{H}$  NMR(200 MHz,  $\text{CDCl}_3$ )  $\delta$  2.55 (3H, s,  $\text{NCH}_3$ ), 3.82 (3H, s, 1- $\text{OCH}_3$ ), 3.85 (3H, s, 10- $\text{OCH}_3$ ), 6.76 (1H, dd,  $J=2.7$ , 8.3 Hz, H-9), 6.87 (1H, d,  $J=8.5$  Hz, H-2), 7.04 (1H, d,  $J=8.5$  Hz, H-3), 7.16 (1H, d,  $J=8.3$  Hz, H-8), 7.89 (1H, d,  $J=2.7$  Hz, H-11);  $^{13}\text{C}$  NMR(50 MHz,  $\text{CDCl}_3$ )  $\delta$  28.3 (t), 33.6 (t), 43.7 (q), 53.1 (t), 55.3 (q), 55.7 (q), 63.1 (d), 110.9 (d), 112.1 (d), 114.7 (d), 121.9 (s), 125.2 (s), 128.1 (d), 128.3 (s), 128.6 (d), 133.0 (s), 136.2 (s), 155.0 (s), 158.1 (s).

**[0063]** 2. Preparation of 1,10-Dimethoxy-7-oxoaporphine (7), a formula VII compound, wherein  $\text{R}_1 = \text{R}_6 = \text{Me}$ ,  $\text{R}_2 = \text{R}_3 = \text{R}_4 = \text{R}_5 = \text{R}_7 = \text{H}$ .

**[0064]** To a solution of compound (6) (148mg, 0.5mmol) in acetic acid (10ml) was added thallium triacetate (816mg, 2mmol). The reaction mixture was stirred at 70°C for 1 hour and then water (150ml) was added. The solution formed was extracted with chloroform (50ml $\times$ 4). The combined chloroform layers were washed successively with saturated sodium bicarbonate aqueous solution (50ml), 10% sodium hyposulfite aqueous solution (50ml) and water (50ml $\times$ 2), followed by drying with anhydrous sodium sulfate and concentration under reduced pressure. The residue obtained was purified on a silica gel column and eluted with chloroform-methanol (99 : 1) to give 1,10-dimethoxy-7-oxoaporphine (7) (100 mg, 69% yield): m.p. 162-164°C; IR(KBr)  $\nu_{\text{max}}$  2934, 2839, 1644, 1617, 1584, 1540, 1484, 1455, 1406, 1373, 1351, 1325, 1255, 1169, 1119, 1048, 1025,

985, 859, 810  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3\text{-CD}_3\text{OD}$ ,  $\delta_{\text{CHCl}_3}$  7.24)  $\delta$  3.79 (3H, s, 10- $\text{OCH}_3$ ), 4.04 (3H, s, 1- $\text{OCH}_3$ ), 6.88 (1H, dd,  $J=2.4, 8.8$  Hz, H-9), 7.47 (1H, d,  $J=9.2$  Hz, H-2), 7.73 (1H, d,  $J=4.7$  Hz, H-4), 7.77 (1H, d,  $J=9.2$  Hz, H-3), 8.29 (1H, d,  $J=8.8$  Hz, H-8), 8.35 (1H, d,  $J=2.4$  Hz, H-11), 8.64 (1H, d,  $J=4.7$  Hz, H-5);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3\text{-CD}_3\text{OD}$ ,  $\delta_{\text{CHCl}_3}$  77.0)  $\delta$  55.2 (q), 56.3 (q), 112.3 (s), 113.5 (d), 113.8 (d), 119.7(d), 124.9 (d), 125.0 (s), 125.9 (s), 130.7 (d), 130.8 (d), 132.3 (s), 136.6 (s), 141.8 (d), 144.6 (s), 159.0 (s), 164.2 (s), 180.7 (s); ESI MS (positive):  $[\text{M}+\text{Na}]^+$  314.

**[0065]** The invention provides aporphine and oxoaporphine compounds for preventing or treating ischemic diseases by maintaining or enhancing the endothelial nitric oxide synthase (eNOS) activity. These compounds can thereby prevent or treat ischemic diseases by maintaining or improving eNOS action. There are no significant side effects to the patients during the treatment of the ischemic diseases with the compounds. Typical side effects may include, for example, memory loss, body temperature decrease etc. Thus, compounds of the invention produce excellent results. When compared with conventional methods in the art for opening up blood vessel by lysing the infarcted thrombus, compounds of the present invention have better therapeutic efficacies by loosening and dilating blood vessels.

**[0066]** While the invention has been described with respect to a limited number of embodiments, those skilled in the art, having benefit of this disclosure, will appreciate that other embodiments can be devised which do not depart from the scope of the invention as disclosed herein. Accordingly, the scope of the invention should be limited only by the attached claims.